

5 **Claims**

1. A method for assaying tropolone or a derivative thereof from animal cell culture supernatant or a proteinaceous solution containing an enriched product protein,
10 comprising the steps of
 - a. Separating the tropolone or its derivative from protein and, prior to that step or after that step,
 - b. Complexing the tropolone or its derivative with Cu(II)-ions in solution
 - c. Assaying tropolone or its derivative by means of reverse phase HPLC with
15 a hydrophobic stationary phase and a mobile phase which mobile phase comprises both Cu(II) ions and an ion-pairing reagent.
2. Method according to claim 1, characterised in that the hydrophobic stationary phase is an alkyl-silane stationary phase.
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3. Method according to claim 2, characterised in that the alkyl-silane stationary phase is an unbranched alkyl-silane phase, preferably is a C-18 alkyl-silane.
4. Method according to claim 1, characterised in that the ion-pairing reagent is an ion-
25 pairing carboxylic acid, or a salt thereof, that is more hydrophobic than trifluoroacetic acid.
5. Method according to claim 4, characterised in that the ion-pairing reagent has a dielectric constant that is equal to the dielectric constants of methylsulphonic acid
30 or hexylsulphonic acid or is in the range defined by the dielectric constants of methylsulphonic acid and hexanesulphonic acid, and preferably is selected from the group consisting of propyl-sulphonic acid, butylsulphonic acid, pentylsulphonic acid, hexansulphonic acid and salts of thereof.
- 35 6. Method according to claim 11 or 1, characterised in that the mobile phase comprises 1 to 30 % of acetonitrile in admixture with at least one further polar solvent.

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7. Method according to claim 6, characterised in the the polar solvent is water, methanol, ethanol or an admixture thereof, preferably the mobile phase comprises at least 60% water.

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8. Method according to claim 1, characterised in that the separation from protein is achieved by firstly precipitating tropolone or its derivative with CuSO_4 and recovering the precipitate and secondly removing protein from the recovered precipitate by ultrafiltration.

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9. Method according to claim 1 or 8, characterized in that the mobile phase comprises CuSO_4 as a source of Cu(II) ions.

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10. Method according to claim 9, characterized in that the concentration of CuSO_4 in the mobile phase is in the range of 0.05 % (w/v) to 0.2 % (w/v).

11. Method according to claim 1 or 10, characterised in the the mobile phase is water-miscible.

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12. Method according to claim 1, characterised in that the supernatant or proteinaceous solution comprising protein is enriched to a concentration of 1 mg/ml or higher.

13. Method according to claim 12, characterised in that the enriched protein is a product protein.

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